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Ultrastructure of the rat hippocampus after isobaric respirative hyperoxia

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Summary. After exposing rats to an environment of isobaric hyperoxia, the ultrastructural alterations of the hippocampus were studied. No major alterations were found in the nerve cells. Of importance was the moderate osmiophilia and the spindle-like transformation of the mitochondria. Vacuolated synapses and neuraxons were found, containing amorphous material. Astrocytic perivascular end feet were found vacuolated in many places. Many endothelial cells of the capillaries presented high osmiophilia, which sometimes prevented structural details. Quantitatively, the findings were proportionally related to the time of exposure in the pure oxygen atmosphere (24, 48 and 65 hours).

Key words: Hyperoxia, Hippocampus, Ultrastructure

Introduction

The deleterious effects of isobaric hyperoxia on the human body have been long known (Dickens, 1945) and consist of easily recognizable nosological entities.

Various experimental works have proved that inhalation of isobaric oxygen, as well as the local action of its free radicals, is responsible for inducing pathological effects, in which the main histological alteration is located in the endothelial cell, being manifested as acute inflammation, ischaemia, hypertension, and syndrome of adult respiratory difficulty (Del Maestro et al., 1981a,b; Rosenblum, 1983; Wei et al., 1985; Olesen, 1987; Unterberg et al., 1988).

Equally well known, also, are the effects of the hyperbaric oxygenation on electrical reaction and on the metabolism of the brain (Torbati and Lambertsen, 1985).

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Various authors in previous studies have experimentally proved that hyperoxia causes alterations on the nerve tissue and that it reduces the electrical resistance of the endothelium of the small brain vessels, resulting in the increase in the permeability of their walls (Olesen, 1987).

In this work, the ultrastructural alterations which were caused by isobaric administration of oxygen on the hippocampus of rats were studied, aiming at ultrastructurally exploring the toxic action of isobaric hyperoxia in the brain tissue.

Materials and methods

Fourteen adult male Wistar rats were used in total. Twelve were divided into 3 groups (A, B, C) of 4 animals each. In every group, 100% of O_2 was administered for 24, 48 and 65 hours respectively, with a flux of 100ml/min. Oxygen was given through a pipe which was inserted into an orifice in the lower part of the animal cage. The circumference of the orifice, outside the pipe, was airtightly sealed. Excess oxygen escaped through thin slots in the upper part of the cage. Soda lime and calcium chloride were placed within the cage to absorb the carbon dioxide and the humidity. Thus, the density of the oxygen inhaled by the animals was 95-100%.

The measuring of the blood gases of the animals was accomplished through blood specimens taken by catheterization of the carotid arteries. The mean value of the partial pressure of O_2 and CO_2 was 301.2 ± 47.77 and 43.12 ± 11.33 respectively.

Two rats, used as controls, were put in similar cages for 65 hours, but were not subjected in hyperoxia.

After instant death of the animals, pieces of the hippocampus were fixed in 3% glutaraldehyde (phosphate-buffered, pH 7.3) for 2 hours preserved on ice, and then they were postfixed in 2% osmium tetroxide (phosphate-buffered pH 7.3). After tissue staining with 1% aqueous solution of uranyl acetate, the tissue pieces were dehydrated in a series of alcohol solutions and then embedded in Epon. Thin sections

were stained with lead citrate and were observed in a Jeol 100CX TEM, at 80kv.

Results

The ultrastructural appearance of the sections taken from the control animals presented no alterations or deviations from the normal ultrastructural pattern.

In all the groups of the experimental animals, there were no remarkable alterations in the nerve cells (Fig. 1). In certain areas, not related topographically, and mostly in the B, C groups, there were some neuraxons and, rarely, nerve cells, presenting swollen or sometimes fully oedematous mitochondria (Fig. 2). Of interest was the presence of mitochondria in the B, C groups, which presented a spindle-like transformation and mediocre to heavy osmiophilia (Figs. 3, 5).

Another finding, presenting a progressive increase in the A, B, C groups respectively, was the presence of rarefactive neuraxons and of some synapses, which contained membranous microcystic or amorphous material (Figs. 4, 5).

More characteristic were the alterations in the network of the vessels. In many areas, the astrocytic end feet on the capillary wall, were less dense or completely destroyed, while many of the endothelial cells presented an impressive osmiophilia. In the animals of the C group, osmiophilia of the endothelium was so intense that the distinction of the structural details of the endothelial cells was very difficult or impossible (Figs. 6, 7).

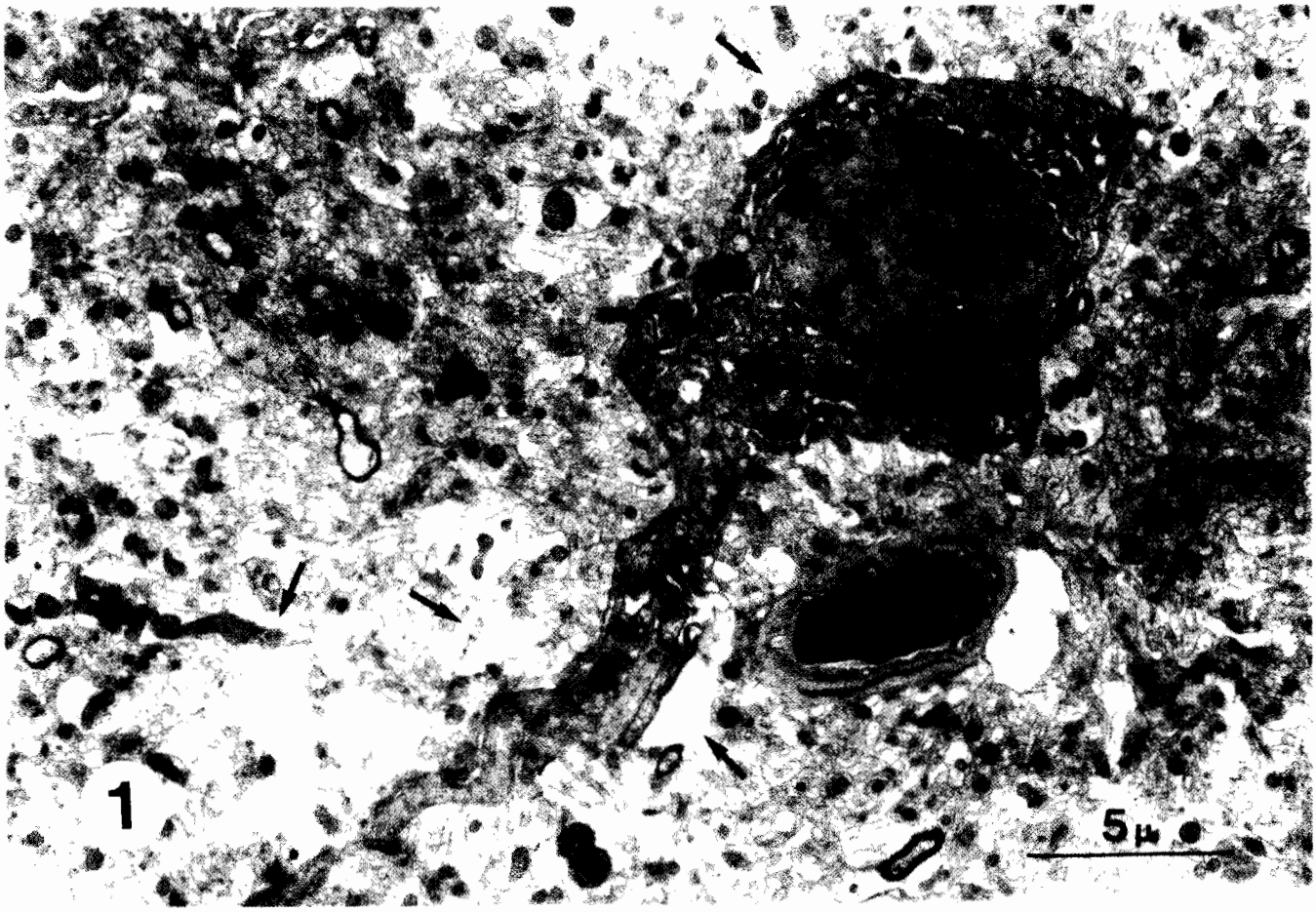
Discussion

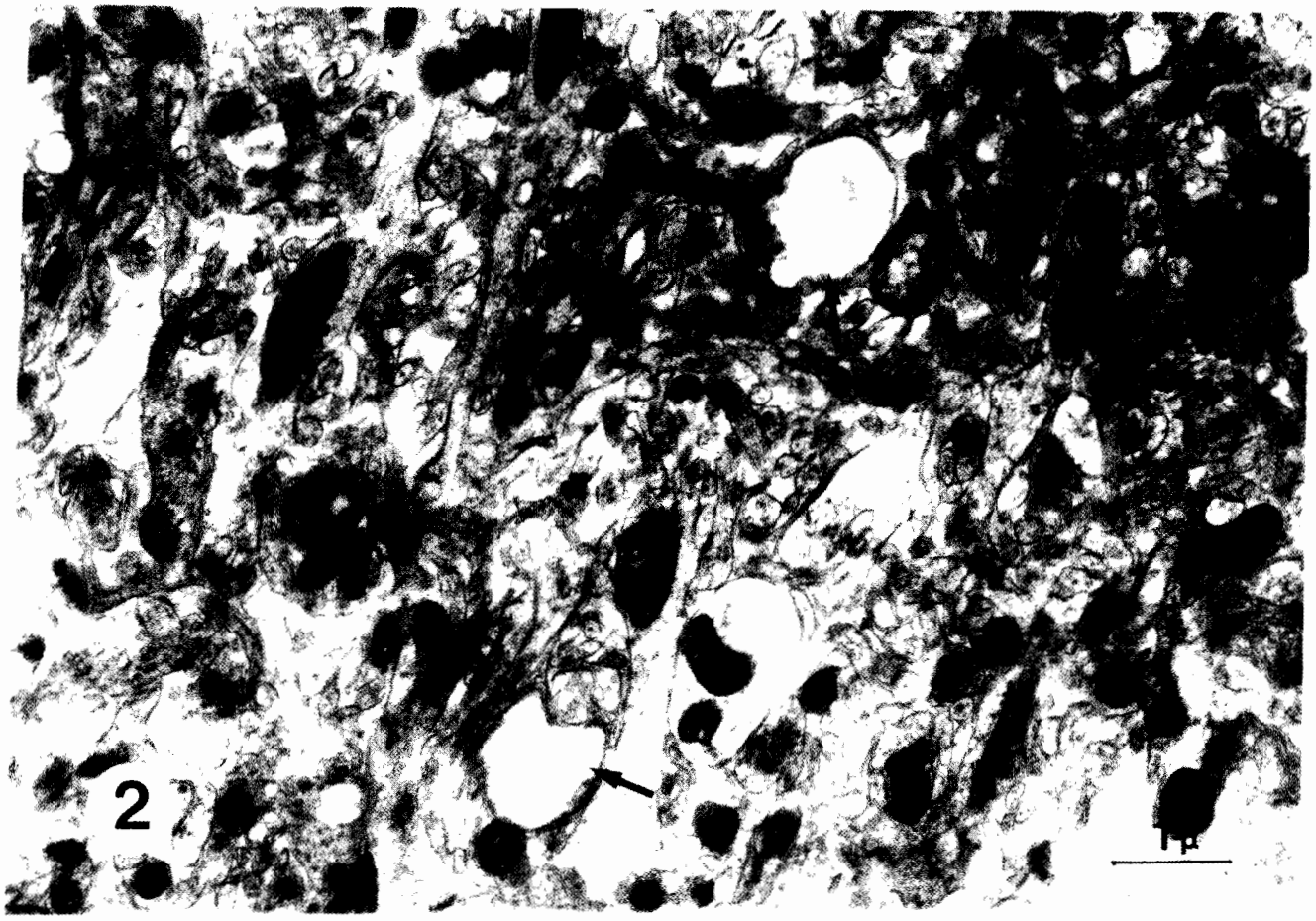
The histological findings of this experimental work, support the view that isobaric hyperoxia is harmful on the CNS. The extent of the various alterations, observed in specimens from the hippocampus, was proportional to the time of the hyperoxia exposure of the animals. Nerve cells seem to be least affected.

The most sensitive of the cell organelles in hyperoxia, are mitochondria. Accumulation of intracellular fluid within the mitochondria and, mainly, the oedema, were the main observations reported by Chan et al. (1982), after incubation of brain tissue along with the presence of free oxygen radicals.

The histological alterations which were observed in this study, in the synapses and in the neuraxons, may well be correlated with the appearance of electrophysiological changes induced by hyperoxia. King and Parmentier (1983) pointed out the existence of reduced presynaptic and post-synaptic action, after exposing the experimental animals in hyperbaric oxygen.

The fundamental mechanism by which O_2 causes these alterations, according to Kovachich et al. (1981), is

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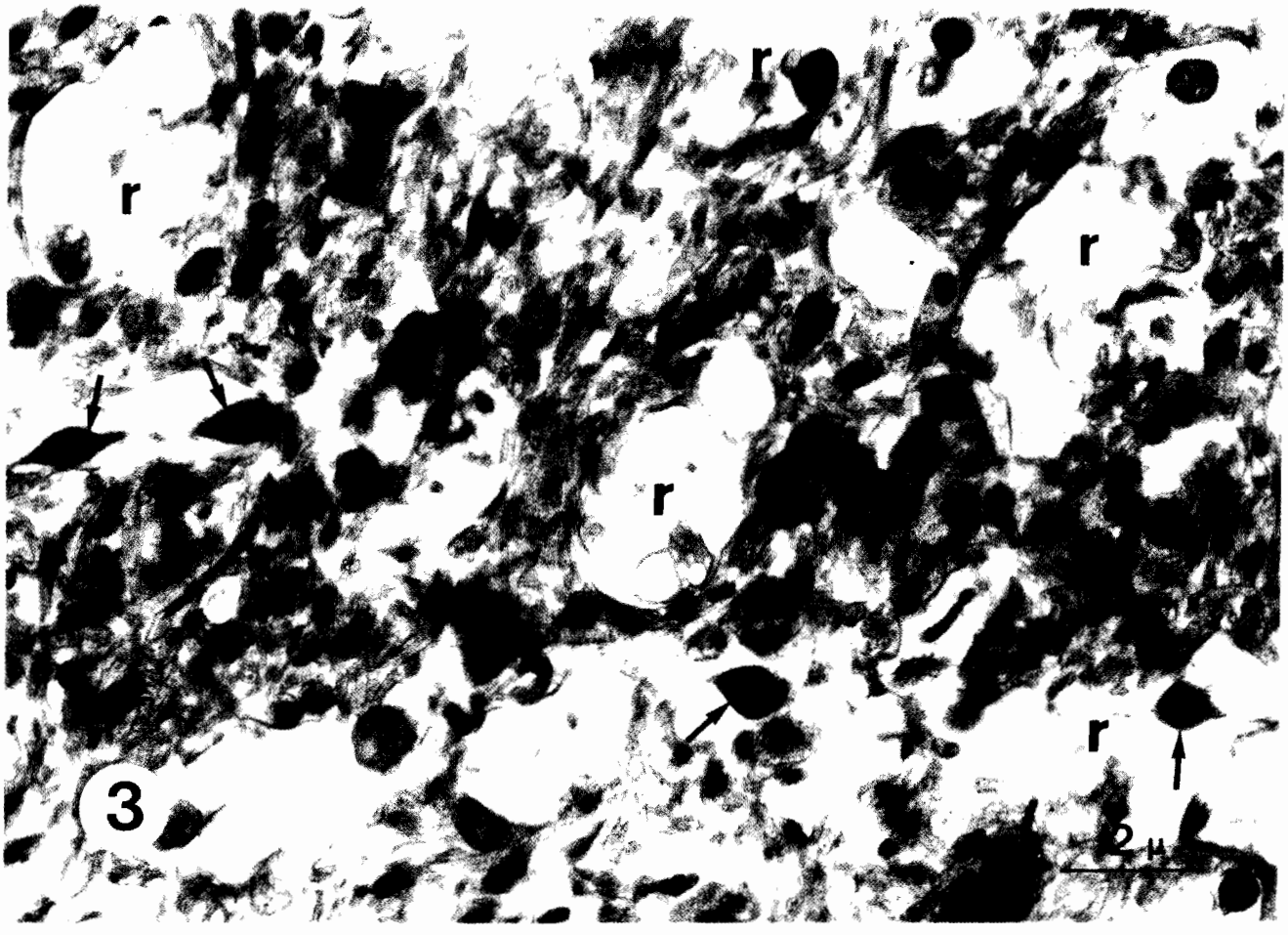
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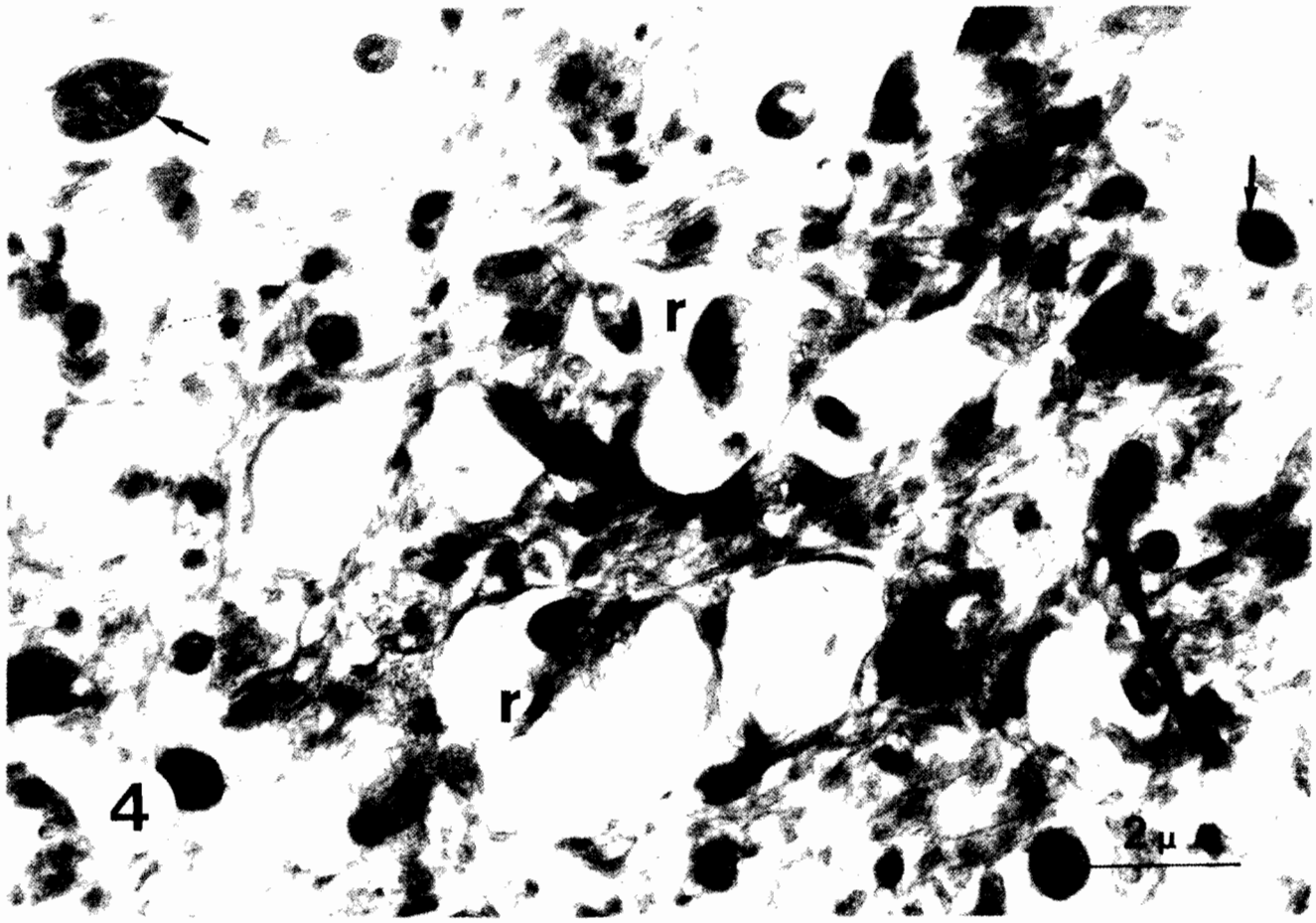
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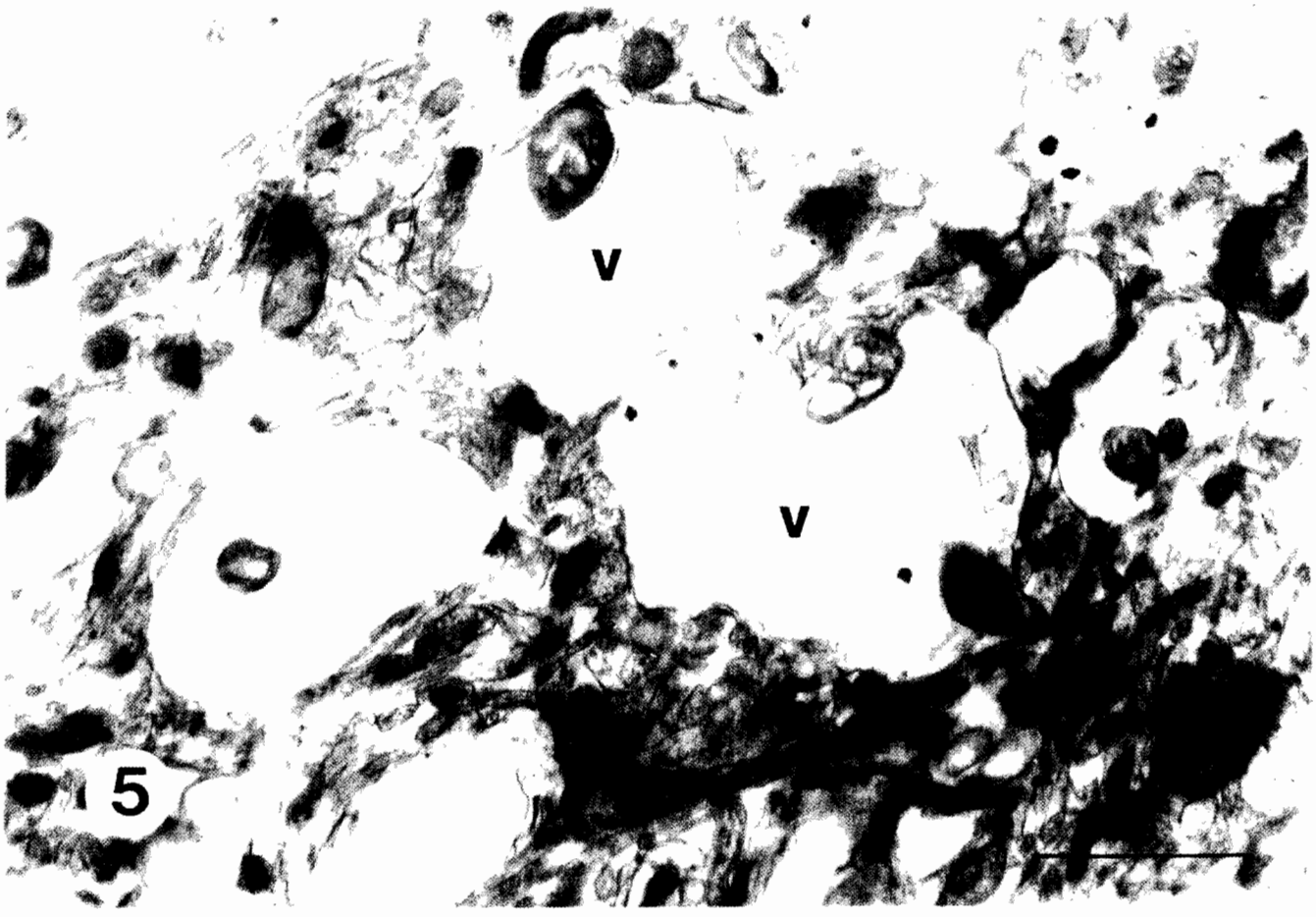
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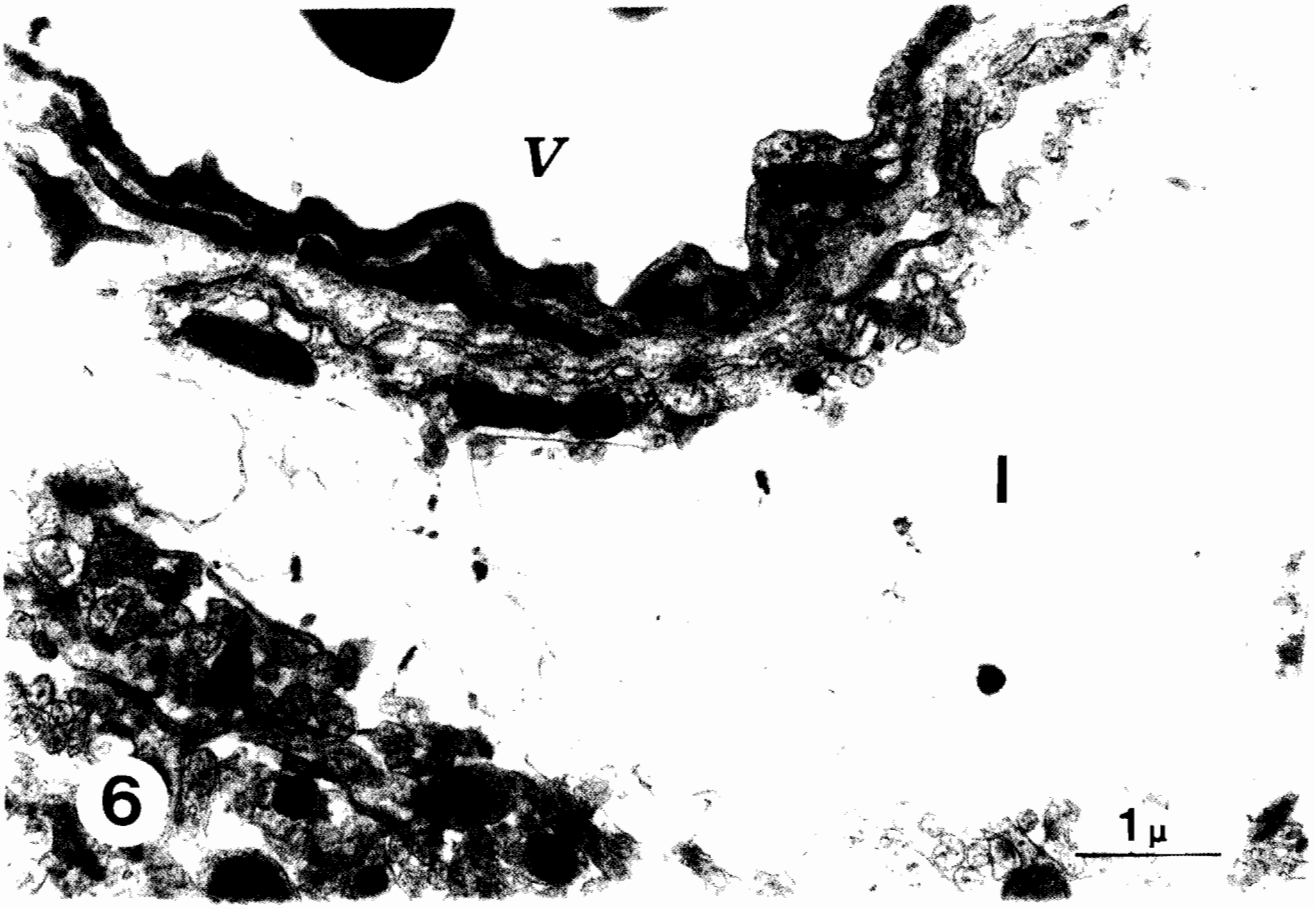
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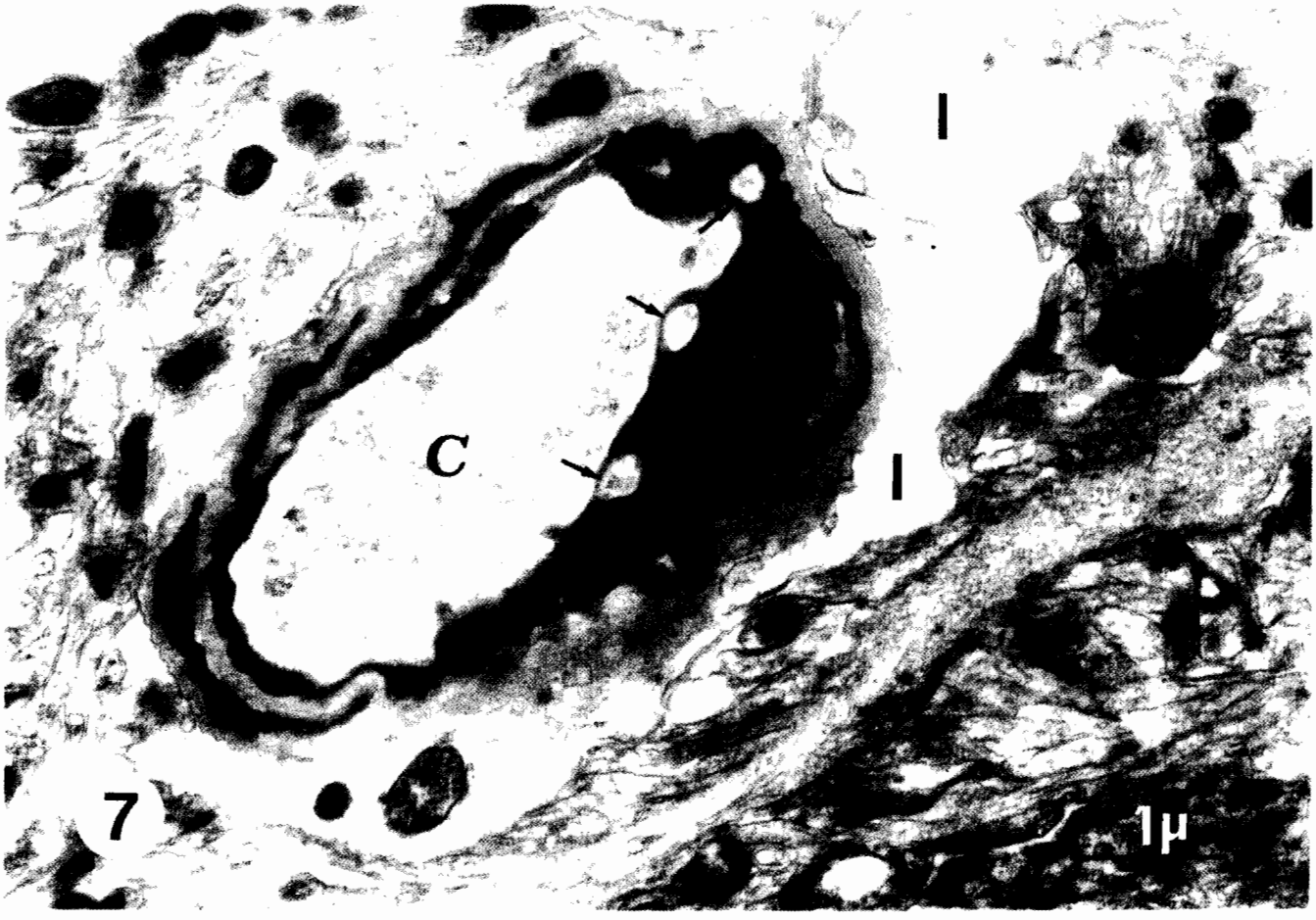
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Fig. 1. 24 hours of oxygen exposure. Gross normal appearance. Some rarefactive areas are shown by the arrows. × 6,500

Fig. 2. 48 hours of oxygen exposure. Arrows show oedema or vacuolation of mitochondria. × 15,000

Fig. 3. 65 hours of oxygen exposure. Spindle-like and osmiophilic appearance of mitochondria (arrows). (r) shows rarefactive areas containing membranous or microvesicular material. × 10,000

Fig. 4. 48 hours of oxygen exposure. Rarefactive areas (r) containing microvesicular or amorphous material. Arrows show spindle-like transformed mitochondria. × 11,500

Fig. 5. 65 hours of oxygen exposure. Greatly vacuolated areas (v). × 14,500

Fig. 6. 48 hours of oxygen exposure. Lytic area (l) adjacent to a small vessel (v) × 18,000

Fig. 7. 65 hours of oxygen exposure. Lytic area (l) adjacent to a capillary (C) apparently of astrocytic end feet origin. The endothelial cells of the capillary are highly osmiophilic and present vacuoles probably of mitochondrial origin (arrows). × 20,000

the depression of Na^+ and K^+–ATPase activity in the brain, resulting in the release of K^+ from the cells and its subsequent accumulation in the extracellular space. This change in K^+ distribution, creates conditions of increased irritability: depolarisation of the pre- and metasynaptic elements.

In this morphological work, the most remarkable indications of the toxic effect of hyperoxia, were the alterations of the endothelial cells of the brain capillary vessels. Similar findings were reported by Wei et al. (1985) in the brain of experimental animals, after local application of an enzymatic system which produced free oxygen radicals. These free radicals, according to the authors' observation, caused functional disturbances resulting in vasodilatation and in the increase of the permeability of the endothelium, which presented structural deviations. The examination of the wall of the functionally inefficient arterioles in TEM, showed oedematous endothelial cells, localized lesions in their cellular membranes and an increased electron density in the smooth muscle cells, sometimes vacuolation, and, rarely, necrosis. Olesen (1987) observed functional disturbances and at the same time selective toxic action of the free oxygen radicals on the cellular membrane of the endothelial cells. The author suggests a lesion of the endothelial glycocalyx, resulting in an influx of a great amount of Ca^{++} within the cytoplasm, as well as in a reduction of the electrical resistance of the capillaries.

The intense osmiophilia of the endothelial cells, mostly in the C group, may be attributed to this increase of the intracellular Ca^{++} which, according to Olesen's observations, derives either from extracellular influx or from the release of the intracellular reservoir in the endoplasmic reticulum. The degree of osmiophilia, as was proven, has an immediate relation to the duration of oxygen inhalation and the lesions that are produced may be permanent in cases of heavy hyperoxia. The lesions of the astrocytes and, mainly, of their end feet, have not been reported previously, and our findings show that this fact can be added to the morphological alterations caused by hyperoxia on the nervous tissue. This lesion seems to be related to the dysfunction of blood-brain barrier and the reduction of the electrical resistance which was observed by Olesen (1987) and Unterberg et al. (1988), after exposure of the brain to the action of free oxygen radicals.

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